PERINATAL JOURNAL

Original Article

Perinatal Journal 2026; 34(2):166-177

https://doi.org/10.57239/prn.26.03420016

Comparative analysis of ovulation triggering strategies on ovarian response, and endometrial receptivity and clinical outcomes in ICSI cycles: Dual trigger VS FSH Co-Trigger VS HCG

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Abstract

It is imperative to optimize ovulation trigger strategies to elevate oocyte maturity, endometrial receptivity and pregnancy in ICSI cycles. Dual trigger (GnRH agonist and hCG) and FSH co-trigger are more physiological options that have been proposed to replace the conventional hCG. The purpose of the present study was to compare dual trigger, FSH co-trigger and conventional hCG trigger protocols during the ICSI cycles with respect to ovarian response, endometrial receptivity as well as clinical outcomes such as fertilization, clinical pregnancy and implantation rates. This is a prospective comparative randomized study that included 102 infertile women who had controlled ovarian stimulation using GnRH antagonist protocol at the Higher Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University. There was a similarity in baseline demographic characteristics and infertility profiles among the study groups. Outcome measures were parameters of ovarian responses (oocyte retrieval rate, oocyte maturity, fertilization, outcomes) and receptivity parameters (endometrial thickness, sub-endometrial Doppler indices) assessed on the day of oocyte pick-up and at the time of embryo transfer and(pregnancy ,implantation rates) The dual trigger and FSH co-trigger conditions had a significantly higher rate of oocyte retrieval, metaphase II (MII) oocytes, oocyte maturation rate and 2PN pronuclei Zygotes, than the traditional hCG group. Both improved triggering protocols were also linked with increased endometrial thickness and stronger changes of uterine and sub-endometrial resistance and pulsatility indices between the triggering day and embryo transfer, which shows better uterine perfusion. Not all of the intergroup differences were statistically significant even though the trend in the dual trigger group had the most desirable result to achieve elevated rates of implantation and clinical pregnancy. Dual and FSH trigger protocols showed an improved performance over the traditional, hCG triggering protocols regarding oocyte retrieval rate, total numbers of mature (MII) oocytes, oocyte maturation rate, normal fertilization (2PN), pregnancy rate and endometrial receptivity indices.

Keywords: Trigger, FSH Co dual trigger, Endometrial receptivity, Oocyte maturation and ICSI

Introduction

Ovulation is the final stage of a complicated process of biochemical and structural activities in the preovulatory follicle, which eventually results in the release of a full-fledged oocyte that can fertilize. Gonadotropins, steroid hormones, growth factors and local mediators in the ovarian microenvironment coordinate this process. [1–3]. In natural cycle, the Luteinizing Hormone(LH) surge accompanied by Follicle Stimulating Hormone(FSH) surge, final maturation of oocytes, cumulus expansion, and follicular rupture occur. [4–6]. In assisted reproductive technology (ART) the ovulation trigger is employed to replicate these physiological responses and to guarantee functionality (i.e., recovery) of metaphase II (MII) oocytes. Human chorionic gonadotropin (hCG) is an established standard because it behaves like LH but the absence of a concomitant rise in FSH and long-term luteotropic effect augments the risk of Ovarian Hyperstimulation Syndrome (OHSS). [7] 10. Recent evidence indicates that dual triggering with a GnRH agonist and hCG improves an oocyte cytoplasmic maturation, cumulus growth and luteal functioning due to a concomitant endogenous upsurge of LH and FSH, which is additional to hCG. [11–14]. additionaly, FSH co-trigger regimens have been introduced to specifically supplement the essential peri-ovulatory FSH rise, which contributes to LH receptor development and maturation competence. [15–17]. Clinical data of the comparative effectiveness of these emerging strategies of trigger proliferation on oocyte maturity, embryo competence and implantation rates are inconsistent despite the rising use of these new strategies. Consequently, this paper seeks to compare clinical outcomes of dual trigger, FSH co-trigger, and standard hCG trigger in women who underwent the ICSI cycles.

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Materials and Methods

It was a prospective comparative clinical trial study that was undertaken at the Higher Institute of Infertility Diagnosis and Assisted Reproductive Techniques, Al-Nahrain University, Baghdad, Iraq, between November 2022 and June 2025. All the women included were subjected to Controlled Ovarian Stimulation (COS) and Intracytoplasmic Sperm Injection (ICSI). All the participants were enrolled on a study protocol that was reviewed and approved by the Local Medical Ethical Committee of the Higher Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University (Approval code: 0701-PF-2023S23) and informed consent to take part in the study was obtained in writing.

A total of 102 infertile women scheduled for ICSI cycles were employed after exclusion of cycles with incomplete data, all women underwent COS using a flexible GnRH antagonist protocol, followed by one of three ovulation-triggering protocols:

- Dual trigger group (n = 39); hCG plus GnRH agonist
- FSH co-trigger group (n = 31); r-hFSH plus hCG
- Conventional hCG trigger group (n = 32); hCG only

The purpose of the study was to compare the ovarian response, endometrial receptivity and the clinical outcome of these three regimens of triggering.

Inclusion criteria

- The qualification criteria were as follows: Women had to fulfill the following criteria:
- Age between 18 and 40 years.
- Body mass index (BMI) 18.5–30 kg/m^2.
- Serum anti-Mullerian hormone (AMH) 1.1ng/mL and higher.
- E2 level of < 2000 pg/mL on ovulation trigger day.
- Indication for ICSI due to:
- Female factor infertility (tubal factor, anovulation, mild endometriosis, or unexplained infertility), and/or
- Male factor infertility
- Normal uterine cavity with no congenital malformations of the uterus and no major intrauterine pathology (polyps, submucous fibroids) diagnosed by clinical and ultrasound studies.

Exclusion criteria

- Patients were not included with:
- E2 level (on trigger day) more than 2000 pg/mL (risk of OHSS and cycle cancellation).
- Hereditary defects of the reproductive tract or of considerable intra uterine pathology.
- Endocrine disorders of the ovarian functioning (e.g., uncontrolled thyroid disease, hyperprolactinemia, inadequately managed diabetes mellitus).
- Hydrosalpinx, or moderate or severe endometriosis.
- Any medical condition that is systemic and is contraindicated by pregnancy or controlled ovarian stimulation.

Materials

Medications, hormonal assay kits, culture media, devices and equipment utilized in this study were those that are used on a regular basis in the Institute. Briefly:

- Stimulation and trigger drugs: recombinant FSH (GONAL-f), HMG (Menogon) GnRH antagonist (Cetrotide), GnRH agonist (Decapeptyl), and hCG (Ovitrelle).
- Hormone analysis: FSH, LH, E2, P4, AMH, and TSH were analyzed by means of Roche kits in the Cobas e411 analyzer.
- Culture media and IVF lab supplies: FertiPro NV (Belgium) fertilization and culture media, flushing and aspiration, mineral oil, PVP, hyaluronidase.
- Equipment: Voluson 2 vaginal ultrasound system, micromanipulation system ICSI, incubators, centrifuges, micropipettes and regular IVF disposables.

Clinical evaluation

The evaluation of all the couples was based on a standardized infertility work-up:

- History Age, infertility duration and type, menstrual patterns, any prior pregnancies or miscarriages, any past infertility treatments, medical history and surgery, medications, smoking and other lifestyle issues.
- Physical examination: vital signs, anthropometrical measurements, hyperandrogenism signs, and thyroid.
- BMI: weight/height 2: weighing 50 kg/height 2 m 2, weight 60 kg/height 2 m 2, weight 70 kg/height 2 m 2.
- Abdominal and pelvic check: to identify any scar, vaginal, cervical, uterine, or adnexal lesions, were excluded by speculum and bimanual examination.

Laboratory investigations

Female hormonal profile

- On cycle day 2, venous blood samples were collected for baseline FSH, LH, E2, AMH, prolactin, and TSH.
- On the day of ovulation trigger, serum E2 was measured; cycles with E2 ≥ 2000 pg/mL were excluded from the study.
- On the day of oocyte pick-up (OPU), serum LH, FSH, and E2 levels were reassessed.
- On the day of embryo transfer (ET), serum FSH, LH, E2, and progesterone were measured.

Blood samples were collected into plain gel tubes, allowed to clot at 37 °C for 30 minutes, centrifuged at 5000 rpm for 5 minutes, and serum was stored at −4 °C until analysis on the Cobas e411 analyzer.

Male evaluation

Semen analysis was performed according to WHO standards to evaluate sperm concentration, motility, and morphology. Viral screening was carried out for both partners according to institutional protocols.

Controlled ovarian stimulation protocol

All women underwent COS using a flexible GnRH antagonist protocol:

- Recombinant FSH (GONAL-f®) was initiated subcutaneously on cycle day 2, with individual dosing based on age, BMI, antral follicle count (AFC), and previous ovarian response.
- In selected cases, HMG (Menogon™) was added to enhance follicular development.
- When leading follicles reached ≥14 mm in diameter, a GnRH antagonist (Cetrotide® 0.25 mg/day) was introduced and continued until the day of trigger.
- Follicular growth and endometrial development were monitored by serial transvaginal ultrasound (TVUS), and gonadotropin doses were adjusted as clinically indicated.

Ovulation-triggering strategies and group allocation

Ovulation triggering was performed when ≥3 follicles ≥17 mm in diameter were present and serum E2 < 2000 pg/mL. Women were randomly allocated to one of three trigger regimens:

- **Dual trigger group:** combined administration of hCG (Ovitrelle® 250 µg) and GnRH agonist (Decapeptyl® 0.2 mg as two 0.1 mg injections).
- **FSH co-trigger group:** administration of recombinant FSH (GONAL-f® 300 IU) together with hCG (Ovitrelle® 250 µg).
- **Conventional hCG trigger group**: administration of two doses of hCG (Ovitrelle®), equivalent to a conventional high-dose hCG trigger.

Oocyte retrieval and ovarian response assessment

Transvaginal ultrasound-guided oocyte retrieval was carried out 34–36 hours' post-trigger under general anesthesia:

- The patient was placed in lithotomy position with an empty bladder; the vagina was prepared with normal saline.
- A single-lumen aspiration needle attached to a suction pump (120–130 mmHg) was used to aspirate follicles from both ovaries.
- The total number of follicles aspirated was

recorded, as well as the total number of oocytes retrieved.

In the embryology laboratory, cumulus–oocyte complexes (COCs) were identified and processed. Ovarian response was characterized by:

- Total number of follicles.
- Number of oocytes retrieved.
- Number of oocytes injected.
- Oocyte retrieval rate (ORR) = (number of oocytes retrieved / total number of follicles) × 100.

Oocyte denudation, grading, and maturity (ovarian response component)

- COCs were incubated and then denuded using hyaluronidase and gentle pipetting.
- Denuded oocytes were classified under an inverted microscope as:
  - Germinal Vesicle (GV),
  - Metaphase I (MI),
  - Metaphase II (MII, mature oocytes with a visible first polar body).

The maturity index (maturation rate) was calculated as:

$$\text{Maturity index (\%)} = \frac{\text{Number of MII oocytes}}{\text{Total number of retrieved oocytes}} \times 100$$

Assessment of endometrial receptivity

Endometrial and sub-endometrial perfusion were evaluated using TVUS and color Doppler at two key time points:

- Day of oocyte pick-up (OPU)
- Day of embryo transfer (ET)

Endometrial thickness

- TVUS was performed with the patient in dorsal lithotomy position.
- Endometrial thickness (EMT) was measured in the mid-sagittal plane as the maximum double-layer thickness from one endometrial–myometrial interface to the opposite interface, excluding intrauterine fluid.
- EMT was documented in millimeters at OPU and ET.

Sub-endometrial blood flow

- Color Doppler mode was activated to visualize sub-endometrial blood flow.
- Pulsatile waveforms were obtained from sub-endometrial vessels; after three consecutive uniform waveforms, the machine automatically calculated:
  - Resistance index (RI)
  - Pulsatility index (PI)
  - Systolic/diastolic (S/D) ratio

Dynamic changes in ET, RI, PI, and S/D from OPU to ET were expressed as percentage change.

ICSI procedure, fertilization, and embryo transfer

Sperm preparation, ICSI micromanipulation, fertilization assessment, embryo culture, and grading were performed according to standard ICSI protocols:

- Fertilization was assessed 16–18 hours after ICSI; normal fertilization was defined as the presence of two pronuclei (2PN) and two polar bodies.

Calculation of fertilization rate was made

Fertilization rate (%)= Number of 2PN oocytes/Number of injected MII oocytes ×100

- Embryos were morphologically evaluated on days 2 and 3; high-quality embryos were prioritized for transfer.
- Embryo transfer was done under ultrasound observation with a soft catheter with the count of embryos being decided by the use of age, best embryo, and prior attempts.
- Luteal phase support with vaginal and/or injectable progesterone was initiated on the day of OPU and continued until 14 days after ET, and further prolonged in women with a confirmed pregnancy.

Outcome measures

Clinical outcomes were assessed as follows:

primary outcome

- oocyte retrieval rate
- total numbers of mature (MII) oocytes, oocyte maturation rate
- normal of fertilized oocyte (2PN).

Secondary outcomes

- Endometrial receptivity,
- Pregnancy rate
- Implaqntation rate

Statistical analysis

Analysis of data was done through SPSS. Continuous variables (ovarian response indices, Oocyte retrieval rate, total numbers of mature (MII) oocytes, oocyte maturation rate, normal of fertilized oocyte (2PN), EMT, RI, PI, S/D, percentage changes, implantation rate) were represented as mean/ Standard Deviation (SD) and compared among the three groups through one-way ANOVA. In instances where the general differences were found to be significant, the cases of post-hoc Tukey and Welch tests were used to compare the cases on a pairwise basis. The categorical variables (e.g., pregnancy rate) were given in the form of number (percentage) and compared by the Chi-square or Fisher Exact test, respectively. The p-value of less than 0.05 was a statistically significant value. The three ovulation-trigger groups had similar levels of baseline characteristics of the study population. No statistically significant differences in the age distribution were found between the dual trigger, FSH co-trigger, and conventional groups (p = 0.201), age distribution indicates that most of the participants were within the 25-34 age group which is are in line with the normal age demographics of reproduction. 14.7% of participants were below 25 year of age, 59.8% were between 25 and 34 years of age and 25.5% were above 35 years old. The age distribution was not statistically significant (p = 0.201), but the majority of the age range consisted of 25-34-year-old, and the percentage of women in the normal weight or overweight range was very high, with only a minor fraction of the women being obese. This indicates that there are no important differences in the weight factor in this study (p = 0. 0.170) The type of infertility was also similar with most of the people having primary infertility which is a common occurrence in studies in the field of reproduction. There are diverse causes of infertility with male factors being the most common ones. It is observed that the study groups did not find any statistic significant difference in age, BMI level, type of infertility, cause and duration of infertility. These results reveal that the three groups were well matched in terms of critical variables of demographics and infertility issues at baseline.

Table 1: Comparison among study groups according to general characteristics

Variable	Dual (n = 39)	FSH Co-trigger (n = 31)	Conventional (n = 32)	Total (%) (n = 102)	P-Value *
Age (years)					0.201
< 25	4 (10.3%)	6 (19.4%)	5 (15.6%)	15 (14.7%)	
25 – 34	22 (56.4%)	21 (67.7%)	18 (56.3%)	61 (59.8%)	
≥ 35	13 (33.3%)	4 (12.9%)	9 (28.1%)	26 (25.5%)	
BMI (kg/m^2)					0.170
Normal	23 (59.0%)	20 (64.5%)	16 (50.0%)	59 (57.8%)	
Overweight	14 (35.9%)	11 (35.5%)	16 (50.0%)	41 (40.2%)	
Obese	2 (5.1%)	0 (0%)	0 (0%)	2 (2.0%)	
Types					0.170
Primary	31 (79.5%)	29 (93.5%)	29 (90.6%)	89 (87.3%)	
Secondary	8 (20.5%)	2 (6.5%)	3 (9.4%)	13 (12.7%)	
Causes					0.535
Male factor	13 (33.3%)	17 (54.8%)	13 (40.6%)	43 (42.2%)	
Female	8 (20.5%)	5 (16.1%)	9 (28.1%)	22 (21.6%)	

factor					
Combined	7 (17.9%)	4 (12.9%)	5 (15.6%)	16 (15.7%)	
Unexplained	11 (28.2%)	5 (16.1%)	5 (15.6%)	21 (20.5%)	
Duration					0.070
< 5	22 (56.4%)	20 (64.5%)	13 (40.6%)	55 (53.9%)	
5 – 9	11 (28.2%)	7 (22.6%)	9 (28.1%)	27 (26.5%)	
≥ 10	6 (15.4%)	4 (12.9%)	10 (31.3%)	20 (19.6%)	

* Chi-square test (χ^2) was used. BMI: Body Mass Index

Comparative study of mean ultrasound parameters on the day of picking up oocyte in table (2) indicated a statistically significant difference between the three groups of study. There was a significant inter-group variation in endometrial thickness (EMT) (p = 0.032). The resistance index (RI) between the groups was highly significant (p = 0.00044). The Dual andFSH Co-trigger groups had reduced mean RI values (0.52 0.15 and 0.48 0.13, respectively) than the conventional group (0.65 0.23). On the other hand, there was no significant difference in pulsatility index (PI) and systolic/diastolic (S/D) ratio (p-value of 0.153 and 0.437, respectively).

Table 2: Comparison of mean of ultrasound finding among study groups on day of ova pick up

Variables	Dual (39) (Mean ± SD)	FSH Co-trigger (31) (Mean ± SD)	Conventional (32) (Mean ± SD)	P-Value
Endometrial Thickness (mm) (ET)	8.16 ± 1.08	8.20 ± 1.01	7.51 ± 1.11	0.032
Resistance Index (RI)	0.52 ± 0.15	0.48 ± 0.13	0.65 ± 0.23	0.00044
Pulsatility Index (PI)	0.81 ± 0.22	0.80 ± 0.26	0.71 ± 0.21	0.153
Systolic/Diastolic Ratio (S/D)	1.86 ± 0.45	1.90 ± 0.40	2.01 ± 0.62	0.437

*ANOVA test (two-tailed) was used. SD = Standard Deviation

In table 3 The comparative analysis of the Doppler ultrasound parameters of the three study groups day of (OPU), The results indicated that there were statistically significant differences in the inter-group parameters of Endometrial Thickness (ET) and resistance index (RI) but not pulsatility index (PI) and Systolic/Diastolic (S/D) ratio. The conventional group showed significantly lower Endometrial Thickness (EMT) than the Dual and FSH co-trigger group (p < 0.05), but did not show any statistically significant difference between the two co-trigger groups (Dual and FSH). In terms of Resistance Index (RI), the traditional group had much greater values of poorer endometrial perfusion compared to both the Dual and FSH co-trigger groups with no difference when comparing between the Dual and FSH co-trigger groups.

It was determined that no significant pairwise difference existed in pulsatility index (PI) or Systolic/Diastolic (S/D) ratio since all the corresponding p-values were greater than 0.05.

Table 3: Pairwise comparison of doppler ultrasound findings on the day of oocyte pick-up in study groups

Variable	Group Comparison	P-Value *
Endometrial Thickness (mm)	Dual vs FSH co-trigger	0.874
	Dual vs Conventional	0.022
	FSH co-trigger vs Conventional	0.018
Resistance Index (RI)	Dual vs FSH co-trigger	0.42
	Dual vs Conventional	0.0012
	FSH co-trigger vs Conventional	0.0003
Pulsatility Index (PI)	Dual vs FSH co-trigger	0.902
	Dual vs Conventional	0.734
	FSH co-trigger vs Conventional	0.712
Systolic/Diastolic Ratio (S/D)	Dual vs FSH co-trigger	0.651
	Dual vs Conventional	0.172
	FSH co-trigger vs Conventional	0.139

Statistical test used: Independent-Samples t-Test (Two-Tailed). P < 0.05 considered statistically significant

Table 4, the parameters that were statistically different during the day of embryo transfer included

EMT, RI, and PI, but not S/D. The endometrial thickness (ET) was found to differ significantly between groups (p = 0.011), with the mean values of the Dual andFSH Co-triggergroups being larger (9.76 ± 1.73 mm and 10.05± 1.08 mm respectively) than in theconventiona lgroup (8.44 ± 3.38 mm). Another value of great significance was the resistance index (RI) (p < 0.001). Mean values of the RI of the Dual andFSH Co-triggergroups (0.45 + 0.14 and 0.42 + 0.13) were significantly lower than the traditional group (0.62 + 0.08). The PI index also demonstrated that there was a statistically significant difference between the study groups (p = 0.001) The conventional hCG-only trigger group showed higher PI index, which demonstrates the increased resistance to vascularity and decreased optimal perfusion. On the contrary, PI values were much lower in the Dual and the FSH co-trigger groups. Systolic/Diastolic ratio (S/D), on the other hand, was not significantly different between groups (p = 0.334).

Table 4: Comparison of endometrial parameters among study groups on the day of embryo transfer

Variables	Dual (39) (Mean ± SD)	FSH Co-trigger (31) (Mean ± SD)	Conventional (32) (Mean ± SD)	P-Value
Endometrial Thickness (mm) (ET)	9.76 ± 1.73	10.05 ± 1.08	8.44 ± 3.38	0.011
Resistance Index (RI)	0.45 ± 0.14	0.42 ± 0.13	0.62 ± 0.08	<0.001
Pulsatility Index (PI)	0.61 ± 0.16	0.62 ± 0.15	0.77 ± 0.22	0.001
Systolic/Diastolic Ratio (S/D)	1.83 ± 0.54	1.80 ± 0.55	1.97 ± 0.34	0.334

*ANOVA test (two-tailed) was used. SD = Standard Deviation

Table 5: Pairwise comparison of endometrial parameters among study groups on the day of embryo transfer

Variable	Group Comparison	P-Value (ANOVA)
Endometrial Thickness (ET)	Dual vs FSH co-trigger	0.394
	Dual vs Conventional	0.021
	FSH co-trigger vs Conventional	0.01
Resistance Index (RI)	Dual vs FSH co-trigger	0.357
	Dual vs Conventional	0.001
	FSH co-trigger vs Conventional	0.001
Pulsatility Index (PI)	Dual vs FSH co-trigger	0.660
	Dual vs Conventional	0.001
	FSH co-trigger vs Conventional	0.002
Systolic/Diastolic Ratio (S/D)	Dual vs FSH co-trigger	0.820
	Dual vs Conventional	0.188
	FSH co-trigger vs Conventional	0.148

ANOVA test (two-tailed) and Welch's t-test were used for pairwise comparisons; SD = Standard Deviation

Table 5 in pairwise comparison of endometria parameters among study groups on the day of embryo ransfer, the results showed no significant differences between the two groups, i.e., dual trigger and FSH co-trigger with respect to endometrial thickness (p = 0.394), resistance index (p = 0.357), and pulsatility index (p = 0.660). On the contrary, both improved triggering protocols led to much higher endometrial thickness (p = 0.021 and p = 0.010) and much lower values of RI and PI values than the traditional hCG trigger (all p ≤ 0.002), suggesting that the endometrial perfusion had been improved. No significant differences were found between the systolic/diastolic ratio of the three groups (all p > 0.05).

This table 6 The mean percentage change in the ultrasound parameters between the day of pick-up of the oocyte to the day of embryo transfer in the three study groups are represented in this table 6. The parties to the research indicated positive changes of ultrasound parameters between the day of pick-up of oocyte and the day of embryo transfer. The most substantial improvements were demonstrated in the Dual and FSH Co-triggergroups where the endometrial thickness improved by +17.5% and +20.1, respectively (p = 0.012). The index of resistance decreased (p < 0.001), particularly in the FSH Co-trigger and dual groups to demonstrate an

improved perfusion of the uterus. Pulsatility index also decreased particularly in FSH Co-trigger group (22.5%), it was very great (p = 0.041). Quite on the contrary, the conventional group showed abatement of smaller percentage variation across all parameters

Table 6: Comparison in mean percentage of change in U/S parameters on the day of transfer compared to day of oocyte pick-Up

Variable	Dual (39) (%)	FSH Co-trigger (31)(%)	Conventional (32) (%)	P-value *
Endometrial Thickness (ET)	+17.5%	+20.1%	+10.9%	0.012
Resistance Index (RI)	−13.5%	−12.5%	−4.6%	<0.001
Pulsatility Index (PI)	−14.1%	−22.5%	−4.9%	0.041
Systolic / Diastolic Ratio	-1.61	-5.26	-1.99	<0.001

One-way ANOVA (two tailed) was used in statistical analysis.

A comparison of clinical parameters among study groups, as presented in Table 7 revealed statistically significant differences in the oocyte retrieval rate(p=0.033), number of mature (MII) oocytes (p = 0.003) and the number of two-pronuclear (2PN) oocytes (p = 0.013) among the groups. The FSH co trigger group produced the highest mean number of mature (MII) and 2PN oocytes, compared to the Dual and conventional groups. No statistic significant differences (P ≥ 0.05) among study groups regarding all other clinical parameter.

Table 7: Comparison in clinical parameter among study groups

Clinical Parameters	Dual (39) Mean ± SD	FSH co-trigger (31) Mean ± SD	conventional (32) Mean ± SD	P-Value*
total dose of chorionic gonadotropin (IU)	2034 ± 786	2162 ± 1220	2285 ± 944	0.675
Duration of stimulation (Day)	10.2 ± 0.95	10.8 ± 1.13	10 ± 2.51	0.208
Total No. of Follicles	14.94± 5.58	14.54±5.67	13.25±4.87	0.403
No. of oocyte aspirated	11.3 ± 4.51	11.51 ± 5.48	9.25 ± 3.78	0.097
Total No. of oocyte injection	8.17 ± 3.54	9.22 ± 4.35	7.78 ± 4.44	0.354
No. of (MI) oocyte	1.85 ± 1.38	1.9 ± 0.94	1.9 ± 1.04	0.986
No. of mature (MII) oocyte	7.97 ± 3.35	9.19 ± 4.37	6.12 ± 2.7	0.003
No. of (GV) oocyte	1.69 ± 0.92	1.63 ± 0.83	2.34 ± 3.27	0.427
2 PN	5.87± 2.55	5.93± 2.97	4.18±2.49	0.013
Maturation rate	71.73 ± 17.7	81.19 ± 14.25	67.44 ± 16.3	0.004
Oocyte retrieval rate	76.18 ± 14.2	77.8 ± 15.01	69.48 ± 10.18	0.033
Fertilization rate	75.61 ± 15.52	72.66 ± 25.93	66.52 ± 17.16	0.146

* ANOVA test (two tailed) was used, MI: metaphase I, MII: metaphase II, 2pn: pronuclei, GV: germinal vesicle.

Pairwise comparison of significant clinical parameters among the study groups as shown in table 8 demonstrated that both the Dual trigger and FSH co-trigger protocols outperformed the conventional protocol across multiple clinical outcomes. The number of mature (MII) oocytes, number of fertilized oocytes (2 PN), maturation rate, and oocyte retrieval rate were all significantly higher in the Dual and FSH co- trigger groups compared with the conventional group (p < 0.05). No statistically significant differences were observed between the Dual and FSH & hCG groups, indicating comparable

clinical efficacy between these two groups.

Table 8: Pairwise comparison of significant clinical parameters among study groups

Clinical Parameter	Group Comparison	p-value*
No. of (MII) oocyte	Dual vs FSH co-trigger	0.256
	Dual vs Conventional	0.004
	FSH co-trigger vs Conventional	0.002
2PN	Dual vs FSH Co-trigger	0.94
	Dual vs Conventional	0.009
	FSH Co-trigger vs Conventional	0.006
Maturation rate	Dual vs FSH Co-trigger	0.038
	Dual vs Conventional	0.412
	FSH Co-trigger vs Conventional	0.004
ORR	Dual vs FSH Co-trigger	0.705
	Dual vs Conventional	0.033
	FSH Co-trigger vs Conventional	0.015

*ANOVA with post-hoc Tukey test. $p < 0.05$ was considered statistically significant. Data are presented as mean ± Standard Deviation (SD). Pairwise comparisons were performed using one-way 2PN: pronucleoli ORR: oocyte retriveral rate

Discussion

The current research was an attempt to assess the effects of three methods of ovulation-triggering, including a dual trigger (GnRH The current research was an attempt to assess the effects of three methods of ovulation-triggering, including a dual trigger (GnRH agonist and hCG) and FSH co-trigger, and the traditional hCG on ovarian response, endometrial perfusion, and initial reproductive outcomes during the ICSI cycles. The key parameters of the ovarian response were the oocyte retrieval rate, the quantity of oocytes at metaphase II (MII) maturity, oocyte maturity rate, and the amount of the two-pronucle (2PN) oocytes that were fertilized normally. Moreover, the clinical outcomes on the use of ICSI as the method of oocyte activation, namely, implantation rate and clinical pregnancy rate were assessed and compared across the study groups. The highest mean number of mature (MII) and 2PN oocytes in ICSI cycles was received in the FSH co rigger group. The baseline demographic and infertility-related factors, such as age, BMI, type, and cause of infertility, and the period of infertility were similar between groups implying that the differences in outcomes could probably have been caused by the trigger protocol, rather than by the selection bias of patients. The comparability is necessary since age-related reduction in the quality of oocytes, endocrine and vascular modifications in body mass index, and varying etiologies of infertility may alter the ART rates independently. [1–5]

The physiological surge of LH in the mid-cycle is accompanied by a smaller but distinct surge in FSH that orchestrates the nuclear and cytoplasmic maturation of the oocyte, cumulus expansion, follicular rupture and subsequent formation of the corpus luteum.[1–4] The conventional ART protocols have largely involved the use of exogenous hCG to simulate LH action and induce the final oocyte maturation but the use of hCG did not replicate the physiological FSH surge and produced a prolonged luteotropic effect that is closely associated with OHSS risk. [5–9] This recognition has driven the development of alternative trigger strategies, including GnRH agonist, dual trigger, and FSH co-trigger, which aim to balance efficacy and safety in controlled ovarian stimulation. [5–7, 11–13]

In the current study, both dual trigger and FSH co-trigger were associated with significantly higher numbers of mature (MII) oocytes, higher numbers of normally fertilized (2PN) oocytes, and improved maturation and oocyte-retrieval rates compared with conventional hCG alone. These findings are consistent with accumulating evidence that restoring a more physiological gonadotropin milieu around the time of ovulation enhances oocyte competence.[5,11,12] Dual trigger protocols, by combining GnRH agonist with hCG, induce an endogenous LH and FSH surge alongside exogenous LH-like activity, thereby mimicking the natural mid-cycle cascade more closely than hCG alone.[11,12,18–22] A several number of clinical studies have found superior oocyte-compromising results with dual trigger as compared to conventional hCG, including in the normal responders, poor responders, and patients who have previously had low MII yield, which contributes to

our finding that this method can maximize oocyte competence within a general ICSI population.Our observation that dual trigger yielded superior oocyte-related outcomes over conventional hCG supports these reports and suggests that this approach may optimize oocyte competence in a broad ICSI population.

FSH co-trigger was superior to the conventional hCG only in regard to the MII oocyte number, 2PN oocyte number, maturation rate and oocyte retrieval rate. These findings are in line with mechanistic and clinical evidence indicating a very important role of FSH in the ultimate maturation of oocytes. It has been demonstrated by experimental work that FSH controls the translation of maternal mRNA, mitochondrial dynamics and cytoplasmic maturation of oocytes and cumulus cells, increasing developmental competence. [14,15] Acute FSH stimulated mitochondrial and metabolic alterations in cumulus cell further confirm its role during the peri-ovulatory window.[15] Clinical trials on human beings have shown that FSH use during the hCG trigger can also enhance the percentage of developed oocytes and enhance embryo development and pregnancy in targeted populations.[16,17,26] We find that our results confirm the notion that FSH supplementation of the hCG trigger is useful in comparison with the hCG trigger, and makes the argument that FSH surge is functionally important not only in oocyte maturation, but also in follicular rupture. [14, 16, 17, 26]

In the comparison of the two types of trigger, i.e. dual trigger and FSH co-trigger, the current study was able to find relatively similar results in terms of oocyte maturing and fertilization with no significant results of the two groups in the majority of the essential laboratory parameters. This implies that either of the two methods may succeed in the restoration or replication of the physiological role of FSH during the final oocyte maturation. [14–17, 18–22]. Nevertheless, dual trigger has a unique capability to utilize an endogenous LH and FSH burst through GnRH agonist, along with exogenous hCG, which might benefit particular patient groups in IVF/ICSI such as past low MII yield, poor response, or asynchronous follicular development, as demonstrated by Lu et al. and Eser et al. in suboptimal and poor responders.[18,19] Meta-analytic and randomized data have similarly indicated that dual trigger can enhance clinical outcomes in certain subsets of IVF/ICSI patients compared with hCG alone.[20–22]

The unique strength of the study is that the assessment of endometrial receptivity was conducted in detail based on the serial ultrasound and color Doppler indices. The non-invasive markers endometrial thickness, Resistance Index (RI), and Pulsatility Index (PI) showed that dual trigger and FSH co-trigger developed a much greater endometrial thickness and low Resistance Index (RI) on the day of oocyte pick-up and day of embryo transfer, as compared to conventional hCG. In addition, the percentage change analysis has revealed that dual and FSH co-trigger groups exhibited greater increases in endometrial thickness, as well as stronger losses in RI and PI between pick-up and transfer which is dynamically enhanced uterine perfusion and receptivity. On the other hand, the traditional hCG group had smaller positive variations on these parameters. Such ultrasound results could be defined in the framework of the emerging molecular data that FSH and LH/hCG receptors are expressed in human endometrial tissue and could regulate angiogenesis and receptivity. [24] Sacchi et al. demonstrated functional FSH and LH/hCG receptors in the endometrium, indicating that pre-ovulatory exposure to these two hormones can have a direct effect on local vascular and cellular remodeling Stilley et al. also demonstrated that FSH controls uterine angiogenesis, which confirms a mechanical relationship between FSH signaling and endometrial blood flow and maturation.[25] In this context, the appearance of the better Doppler profile in the dual group and FSH co-trigger group with thicker endometrium and reduced vascular resistance produces a biologically viable outcome and may act as the contribution to the aspects of a better implantation process relative to the standard hCG use.

Although the study was not primarily powered to detect differences in pregnancy and implantation rates, the trends observed favored the dual trigger group, with the FSH co-trigger group also performing better than conventional hCG. These clinical patterns comply with earlier randomized trials and meta-analyses of higher or at least equivalent pregnancy rates with dual trigger relative to hCG alone.[20–22,23]

Hsia et al. and Haas et al. Hsia et al. and Haas et al. had been found to have better or equally good pregnancy rates in fresh IVF cycles using dual trigger and Chen et al. indicated in a meta-analysis that dual trigger would better the oocyte maturity and occasionally the clinical pregnancy rates in chosen groups.[20–22,23] The results of our study add to this body of literature because, at the same time, we were able to show that there are positive results in laboratory results and objective indicators of endometrial perfusion.

The adoption of new trigger strategies is more concerned with safety. The use of conventional high-dose hCG has been proposed as an established cause of OHSS since it may have a prolonged luteotropic effect and owing to its stimulation of VEGF release in luteinized granulosa cells. GnRH agonist trigger with the appropriate luteal support has been suggested as a mechanism to decrease the risk of OHSS without impacting efficacy, as well as dual trigger with reduced dose of hCG.[6,10,11,27,28] While our study excluded women at high risk of OHSS by limiting estradiol levels at trigger, and did not directly compare OHSS incidence among groups, the use of dual trigger and FSH co-trigger may offer theoretical safety advantages when combined with individualized hCG dosing, as suggested by Rosen and Meldrum and by contemporary guidance on GnRH agonist trigger in modern practice.[27,28] Future studies incorporating high-risk populations and detailed OHSS outcomes are warranted to clarify these potential safety benefits.

Altogether, the current results demonstrate the changing idea that ovulation trigger cannot be regarded as merely a binary phenomenon to induce follicular rupture, but rather as a timely trigger to best oocyte competence and endometrial receptivity. Dual trigger seemed to offer the best overall benefit, specifically in oocyte related outcomes and implantation patterns, although FSH co-trigger had considerable benefits too and might be a prospective solution in cases where GnRH agonist cannot be utilized or is contraindicated.

The study has several limitations. It was conducted at a single center with a relatively modest sample size, which may limit the power to detect differences in hard endpoints such as live birth and long-term safety. Only fresh transfer cycles were evaluated, and cumulative outcomes including frozen–thawed transfers were not assessed. Additionally, molecular markers of endometrial receptivity and luteal phase dynamics were not measured, so mechanistic inferences are based on Doppler surrogates and existing literature. [1, 24, 25] Despite these limitations, the consistency of our results with established physiological principles and prior clinical trials strengthens their validity.

In conclusion, this study adds to the growing evidence that advanced ovulation-trigger strategies—particularly dual trigger enhance oocyte retrieval rate, oocyte maturity, fertilization parameters, and endometrial perfusion compared with conventional hCG trigger in ICSI cycles. Integrating dual or FSH co-trigger into individualized COS protocols may help optimize reproductive outcomes while potentially reducing reliance on high-dose hCG and associated OHSS risk.

Acknowledgements

The authors show gratitude to all members of the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies in AL Nahrain University, for their great help and all couples who agree to contribute in the study.

Abbrevation

- (RI) Resistance index
- (PI) Pulsatility index
- (S/D) Systolic/diastolic ratio
- ICSI Intracytoplasmic Sperm Injection
- TVUS Transvaginal ultrasound
- OPU ova-pickup.
- ET Emberyo transfer

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